from those discussed by Prakash and Rao.[5] The system is thought to be too complex, involving at least two components – substrate and enzyme – for us to use it commercially, and single compounds were the basis of the book.[5] The present state of biotechnology may be too primitive to effect the transfer of the genetic systems involved into other plants, in spite of recent wishful thinking.[13] Furthermore, because so many of our food plants are already cyanogenic, it may not be sensible to attempt to transfer cyanogenesis into those few important ones that are not.

The data from a large number of sources show that cyanogenesis appears to have played an essential part in the choice of the major food plants by humans at the time of transition from hunter-gatherer to cultivator and husbandman.[11] The evidence is based on the following observations (see Reference 11 for details): (1) Our food plants have to be ones we can eat in quantity. Most other potential food plants contain compounds that we cannot detoxify or metabolize in quantity. We have found other uses for some of these compounds. In small doses, many are medicinal drugs, although in larger doses they are metabolically dangerous. (2) Our food plants have to be easy to grow with minimal care and attention, and 'good' to eat. (3) The cyanogenesis of the leaves deters many would-be pests. A plant with few pests would be an attractive candidate for domestication by our ancestors. These plants are not difficult to grow as crops and could well have been higher-yielding than other candidates not similarly protected. (4) Given sufficient protein we can detoxify the hydrogen cyanide released by raw cyanogenic plants, so long as they are only part of a meal. (5) By processing the food before eating we can remove most of the hydrogen cyanide and the cyanogenic compounds and so eat much more. Very few other organisms pre-process food, not even our primate relatives.[10] (6) Our domesticated animals can also detoxify hydrogen cyanide, again given sufficient protein in the diet. (7) It is the leaves of our cereal crops that are cyanogenic, not the grains. We eat the grain, our animals may eat the rest. (8) Cyanogenic glycosides and the β-glycosidases are economical compounds for plants to produce; they are turned over in the plant and are used only when the plant is damaged. (9) Although cyanogenic glycosides are arguably the most widespread of all chemical defences in vascular plants and, when degraded, are remarkably effective herbivore deterrents (pesticides), we have included a disproportionally large number of cyanogenic plants among our staple foods.

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Bis-pyrimidylpyrazolinones – a new class of acetohydroxy-acid synthase (AHAS) inhibitor

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Abstract: Hydroxypyrazolinones which bear two pyrimidine rings (on N-1 and C-4) were found to be potent inhibitors of acetohydroxy-acid synthase which displayed good herbicidal activity *in vivo*. Structure–activity relationship studies suggested the presence of a second binding niche on the enzyme for a 4,6-dimethoxypyrimidine ring.

Keywords: acetohydroxy-acid synthase; dimethoxypyrimidine; herbicide; pyrazolinones; structure–activity relationship

1 INTRODUCTION

In random screens, 1-(3,5-dichlorophenyl)-4-isobutylpyrazolidine-3,5-dione was identified as a moderate herbicide lead which induced symptoms on the plants consistent with the inhibition of acetohydroxy-acid synthase (AHAS). Analog synthesis first concentrated on pyrimidine replacements of the phenyl ring. As an unexpected reaction product, a pyrazolinone was obtained which bore pyrimidine rings on N-1 and C-4. Surprisingly, this new compound was significantly more active than previous analogs. A standard enzyme assay confirmed AHAS inhibition as the primary mode of action.

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(*Received 1 July 1998; accepted 1 February 1999*)

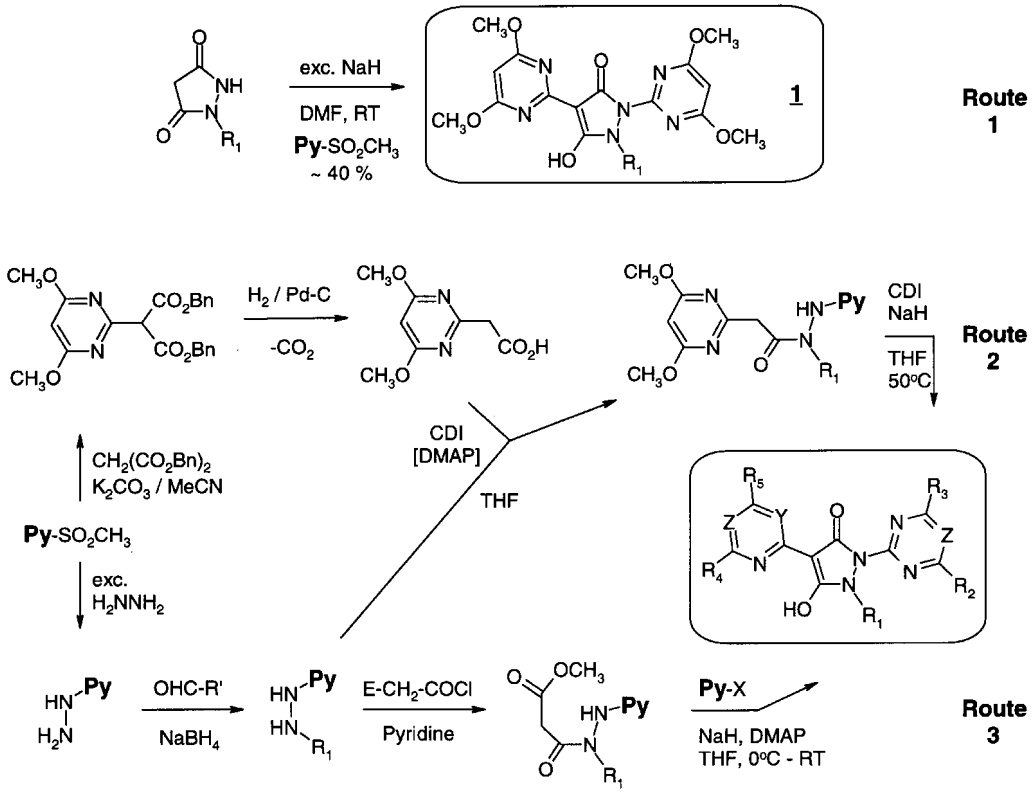

Figure 1. Synthesis routes to pyrazolinones (Py = disubstituted pyrimidine or triazine; Z, Y = CH or N; R_1 = lower alk(en)yl, R_2-R_5 = CH_3, CH_3O, Cl).

2. EXPERIMENTAL AND RESULTS

The routes used for the synthesis of novel bis-pyrimidylpyrazolinones are depicted in Fig 1. Route 1 provided rapid access to analogs with 4,6-dimethoxy substitution in both the pyrimidine rings, but failed with any other substitution pattern or with other azine rings. Neither could the arylation reaction be stopped at the stage of the mono-pyrimidylpyrazolinone. For further investigation of the optimum substitution at N-1 and C-4, different routes for construction of the central pyrazolinone ring had to be developed, which brought in the desired substituents already attached to the building blocks.

The most intriguing question arising from the unusual structure of the lead compound was whether both pyrimidine rings were really necessary for good activity. Replacing either of them by phenyl, alkyl, or hydrogen led to a complete loss of activity. Since sufficient acidity is known to be a prerequisite of AHAS inhibitors, the role of the pyrimidine ring at C-4 was also speculated to be solely one of anion stabilizing rather than binding to the enzyme itself (pKa of **1**, $R_1 = C_2H_5$, is 6.0). Its replacement by other electron-withdrawing groups like ethoxycarbonyl or phenylsulfonyl, however, destroyed all activity. Even the relatively minor change from 4,6-dimethoxypyrimidin-2-yl to 2,6-dimethoxypyrimidin-4-yl at position C-4 resulted in a sharp drop in activity. It was therefore concluded that both pyrimidine rings at N-1 and C-4 did indeed bind to the enzyme, and in a similar fashion structure–activity requirements within both rings were found to be identical and typical for AHAS inhibitors: activity decreased in the order pyrimidines > triazines; and with 4,6-substituents: $CH_3O > Cl > (CH_3)_2N > CH_3 \gg H$, PhO, etc. (In contrast to this, the original lead structure bears only *one* 3,5-dichlorophenyl ring at N-1, yet is active – here, deprotonation probably occurs at N-2 rather than C-4, leading to a different orientation of the anion at the enzyme binding site).

Structural freedom on N-2 was found to be limited. Highest activity was achieved by C_{2-4} -alkyl or allyl substituents: the activity ranking was 2-chloroallyl > 3-chloroallyl > isobutyl, *tert*-butyl, 2-methoxyethyl, 2-cyanoprop-2-yl > H, isopropyl, 2,2,2-trifluoroethyl, 3-methyl-2-butenyl > phenyl, benzyl, neopentyl. As a consequence, the intrinsically most active molecules may have been less lipophilic than desirable for uptake and transport.

A variety of other heterocycles was investigated as a replacement for the central pyrazolinone ring, all featuring two pyrimidine-, a lipophilic alkyl-, and an acidic hydroxy-substituent(s). None of these displayed useful herbicidal activity.

The greenhouse activity of a typical pyrazolinone **1**, R_1 = 2-Cl-allyl, is shown in Table 1.

Generally, activity post-emergence was equal to or slightly better than pre-emergence, and slightly better on grasses than on broad-leafed weeds. The best analogs controlled several species at 50 g ha^{-1}, but required 150–200 g ha^{-1} for acceptable broad-range weed control. At these rates, selectivity was marginal in oil seed rape or cotton and insufficient in economically more important crops.

Table 1. Herbicidal activity of 1 (R=2-Cl-allyl) on grasses 20 days after post-emergence treatment

	Control (%)											
$g\,ha^{-1}$	Maize	Wheat	Barley	Cotton	Signal grass	Bamyard grass	Johnson grass	Green foxtail	Black grass	Wild oat	Annual meadow grass	Yellow nut-sedge
800	92	95	89	89	95	95	89	95	95	95	95	78
200	89	92	89	83	95	94	92	94	86	92	92	42
50	72	83	86	36	83	92	69	92	86	81	92	11

In-vitro inhibition constants were determined for the majority of analogs in a standard assay with partially purified AHAS enzyme from etiolated maize shoots. The best inhibitors achieved IC_{50} values of 0.16 –0.35 μM. This is well within the range of those of commercial herbicides (chlorsulfuron IC_{50} = 8.5 nM; flumetsulam IC_{50} = 0.1 μM; imazethapyr IC_{50} = 3.5 μM). The in-vitro data correlated well with the activity ranking observed in the greenhouse. The kinetics of the enzyme inhibition were studied in more detail for one analog (**1**, R_1 = isobutyl). As with other classes of AHAS inhibitor, a slow tight binding characteristic was found and higher inhibition was measured after 30–40 min incubation.

In a simple qualitative model of binding to AHAS, the pyrimidine rings of different known inhibitors were superimposed. The conformation of the remainder of the molecule (in the anionic form) was then optimized for binding to a putative positive centre which was fixed in space. The results for the pyrazolinones suggest that the pyrimidine ring at N-1 is equivalent to that of other inhibitors. The second pyrimidine at C-4 participates in binding to the positive centre, with the negative charge of the anion being delocalized into the ring.

3. CONCLUSIONS

Although the pyrazolinones did not achieve commercially useful herbicidal activity *in vivo*, they constitute a novel class of intrinsically potent AHAS inhibitors with some unique structural features. SAR suggests the presence of a second binding niche on the enzyme for a 4,6-dimethoxypyrimidine ring, in close proximity to the binding site of the acidic functionality of the inhibitors.

Temperature-insensitive microemulsions and dilution ripening

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(*Received 1 July 1998; accepted 1 February 1999*)

Abstract: Single-phase microemulsions are thermodynamically stable dispersions of an oil in water (O/W) or water in oil (W/O) and are an attractive means of formulating agrochemical products (bicontinuous systems of mutually dispersed oil and water are also possible). Two areas of interest are reported in this summary. (i) Temperature sensitivity – a disadvantage of many microemulsion systems is that the phase boundary of the single-phase region is often highly dependent upon temperature and electrolyte concentration. The aim of this work was the production of temperature-insensitive systems, the criterion used being that the radius of curvature of the droplets at the solubilisation boundary was independent of temperature. (ii) The application of microemulsion systems will involve dilution into water to form the spray system and, as such, an understanding of how the droplets grow under these conditions is of importance. Factors affecting droplet growth processes occurring upon dilution into water have been investigated.

Keywords: microemulsions; temperature sensitivity; dilution ripening

The phase behaviour of microemulsions (and hence solubilisation capacity) is dominated by the curvature of the surfactant layer around the droplets. At the solubilisation phase boundary the curvature of the interface is the 'natural' curvature for that set of experimental conditions, and close to the Phase Inversion Temperature (PIT) this curvature scales linearly with temperature for many surfactants. The natural curvature is determined by the relative sizes of the headgroup and the tail of the surfactant. If these are similar in size, large droplets are formed, whilst increasing the relative size of the headgroup or the tail will result in smaller O/W or W/O droplets respectively.

Plots of the reciprocal of the natural radius (curvature) versus temperature have been determined for heptane/ 60 mM sodium chloride microemulsions stabilised by Aerosol OT (AOT) and dodecyl pentaoxyethylene glycol ether ($C_{12}E_5$) and their mixtures. The radii of curvature were estimated from the extent of solubilisation. All of the plots were linear, with the reciprocal of r_{nat} decreasing with temperature for the non-ionic surfactant and increasing for the ionic surfactant; mixtures showed intermediate slopes. The oppositely signed slopes arise from the fact that increasing temperature shrinks the headgroups of